EFFECTS OF SOME FUNGICIDES ON GERMINATION, GROWTH AND SPORULATION OF *CURVULARIA CYMBOPOGONIS.*

D.B. OLUFOLAJI

Federal University of Technology,
Akure. Nigeria.

ABSTRACT. — Studies were carried out to investigate the efficiency of four fungicides: — Aretan 6 (organomecurial), Benlate (Benlate), Dithane M-45 (Mancozeb) and Tilt (propiconazole), against a strain of *Curvularia cymbopogonis*, causal organism of a leaf spot disease of sugar cane. *In vitro* tests showed these fungicides were toxic to the pathogen at various concentrations and at different developmental stages. Aretan 6 allowed minimum germination only at 1g/l concentration. Tilt and Benlate were also effective at the germination stage since up till 36h after inoculation in 5g/l of the fungicides they prevented germination better than Dithane-M45 which thus proves to be less effective. Aretan inhibited mycelial growth at even lower concentration, while Benlate and Tilt inhibited mycelial growth and sporulation correspondingly at higher concentration: 3-5g/l. However, Dithane-M45 showed no fungicidal or fungistatic activity at the tested concentrations. Finally at 5g/l, Benlate and Tilt, totally inhibited mycelial growth and spore formation in *C. cymbopogonis*.

RÉSUMÉ. — Des études, concernant l'efficacité de quatre fongicides (Aretan 6, Benlate, Dithane-M45 et Tilt) sur le développement du *Curvularia cymbopogonis*, champignon responsable du " leaf spot " de la canne a sucre, ont été entreprises. Les études *in vitro* montrent que ces quatre fongicides sont toxiques pour le *Curvularia cymbopogonis* à des concentrations différentes et à des stades distincts du développement du champignon. A la concentration de 1g/l, l'Aretan 6 présente l'inhibition de germination la plus élevée. Le Tilt et le Benlate sont actifs au stade de la germination jusqu'à 36 heures après l'inoculation à la concentration de 5g/l. Le Dithane-M45 est nettement moins actif. L'Aretan inhibe la croissance à la concentration de 2g/l. Le Benlate et le Tilt nécessitent quant à eux des concentrations plus élevées. Le Dithane-45 n'est pas actif en tant que fongicide. A la concentration de 5g/l, le Benlate et le Tilt arrêtent la croissance et la sporulation de *C. cymbopogonis*.

KEY-WORDS : *Curcularia cymbopogonis*, fungicide, Sugar Cane, Leaf Spot, Germination, growth.

MOTS-CLÉS : *Curvularia cymbopogonis*, fongicides, canne à sucre, germination, croissance.

INTRODUCTION

Some of the common diseases of sugarcane are smut disease caused by *Ustilago scitaminea* Sydow and *Physalospora tucumanensis* Speg. (Antoine, 1961). Parris (1950) also reported eye spot (leaf spot) disease of sugarcane caused by *Helminthosporium sacchari* Butler. In 1986, Olufolaji found *Curvularia cymbopogonis* (C.W. Dodges) Grooves & Skolko to be the causal organism of a new leaf spot disease of sugarcane in Nigeria. This disease was called "Curvularia leaf spot". It is nowadays assuming prominence in Nigerian sugarcane farms and accordingly control measures are needed before it gains economic importance.

In Nigeria several trials have been carried out to control diseases of sugarcane with fungicides (Olufolaji & Olofinboba, 1984; Olufolaji, 1985). These fungicides, are methoxy-ethyl-mercuric chloride 6% Hg (Aretan), triadimefon 25% (Bayleton) and Benomyl 50% (Benlate) which are diverted to control smut and red-rot disease (Olufolaji, 1985).

In Nigeria also, zineb and related fungicides have been tried in controlling "Curvularia leaf spot" disease of maize caused by *C.pallescens* Boed. (Fajemisin & Oyekan,1976). However, there is ■ need to try some of the available fungicides in controlling the new "Curvularia leaf spot" disease of sugarcane in this country.

MATERIALS AND METHODS

Fungicides :

Four fungicides: Aretan 6, Benlate, Dithane-M45 and Tilt were used on *Curvularia cymbopogonis*.

Effects of the fungicides and spore germination :

Exactly 0.05ml of a spore suspension of the fungus was dropped at three spots on clean glass slides. Drops were allowed to be fairly air dried, 0.05ml each of the fungicides concentrations of 1, 2, 3, 4 and 5g/l are dropped on each of the air-dried spore suspension.

A humidity chamber was prepared by placing wet filter papers inside a medium sized petri dish and its lids. Glass slides supported by bits of glass rods were placed inside these petri dishes before incubation at 27°C.

After 24h of incubation, slides were observed for spore germination using a compound Olympus Tokyo microscope. With the aid of a tally counter, germinated and ungerminated spores were counted and percentage germination estimated. The above procedure was carried out for all the fungicides at tested concentrations using 4 replicates. For the control, distilled water was used.

Effects of the fungicides on growth and sporulation:

Exactly 0.05, 0.1, 0.15, 0.20, and 0.25g of each fungicide were dissolved in 50ml each of malt extract agar (MEA). Such weights gives concentrations of 1, 2, 3,

4 and 5g/l respectively. About 12.0ml of the agar-fungicide mixture was poured into 4x12cm medium sized sterile petri dish. Using a sterile cork borer, 0.5cm diameter circles were cut from a week old culture of the *C.cymbopogonis* and each placed upside down onto the centre of the agar in the petri dishes. This procedure was carried out for all tested fungicides at all concentration levels. Inoculated media were incubated at 27°C. A control experiment with MEA without fungicide was inoculated as well. All inoculated plates were observed daily up to 10 days and radial growth measured in diameter (mm).

As regards sporulation, spores were harvested from petri dishes at the 10th day after inoculation. Agar fragments of 1cm2 were cut from the culture surface and dropped in a 25ml beaker containing 5ml distilled water. With the aid of carmel's hair brush, spores on the agar mass surface were dislodged into the water. Spore concentration in the spore suspension was counted with the aid of improved Neubauer's Haemocytometer slide.

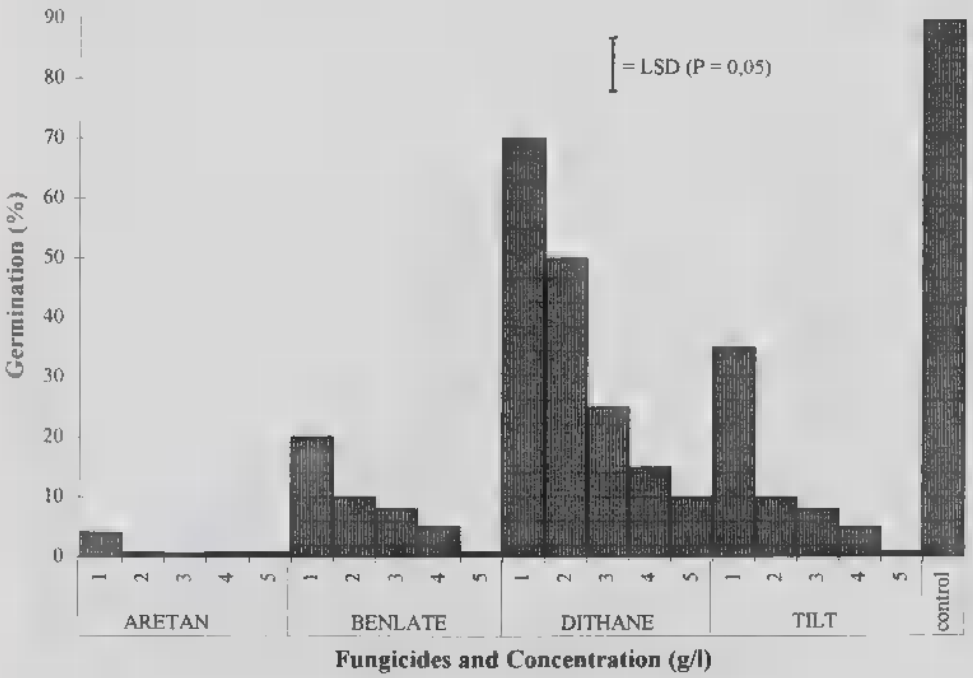

Fig. 1: Germination of spores of *Curvularia cympogonis* on glass slides in differents fungicides concentrations after 36 h of incubation.
Fig. 1: Taux de germination des spores de *Curvularia cympogonis* après 36 h sur lame en présence de différentes concentrations des fongicides étudiés.

RESULTS

Effects of the fungicides ▬ germination of *C. cymbopogonis*:

Results in fig.1 shows that all tested fungicides were active in inhibiting spore germination in *C.cymbopogonis* at the highest experimental concentrations. Aretan only allowed germination at a concentration of 1g/l. Benlate and Tilt had a similar effect on the fungus by dissallowing germination only at 5g/l concentration. However Dithane-m45 exhibited highest germination percentage (70%) at 1g/l and lowest germination (10%) at 5g/l (fig.1).

Effects of the fungicides on mycelial growth of *C. cymbopogonis*. :

Radial growth measurements for the different fungicides are shown in fig.2. Aretan did not allow mycelial growth except at concentration of 1g/l; radial growth recorded was 4mm. Benlate and Tilt showed no mycelial growth at 5g/l. Tilt allowed corresponding more development up to 4g/l with highest growth of 38,37mm observed at 1g/l and lowest of 12mm at 4g/l. At the latter concentration, Benlate had the same effect. Dithane-M45 allowed highest growth of all the fungicides with a maximum of 82mm, at 1g/l and a minimum of 20mm at 5g/l fungicide concentration.(fig.2)

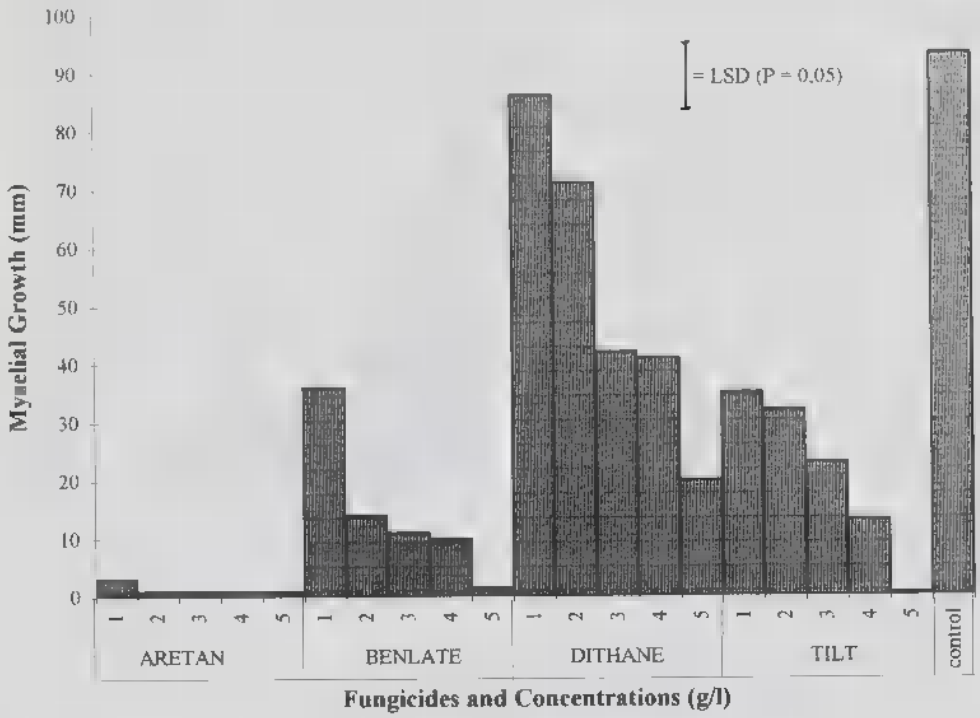

Fig. 2 : The effect of some fungicides at different concentrations on mycelial growth of *Curvularia cymbopogonis*.

Fig. 2 : Croissance de *Curvularia cymbopogonis* en présence de différentes concentrations des fongicides étudiés.

Mycelial growth in the control was profuse and rapid: 90mm growth at the tenth day (fig.2).

Fungicides effects on sporulation of *C. cymbopogonis*:

There was profuse sporulation in the control as opposed to other treatments (Table 1). In all four control replicates an average of 1700 spores were produced. Aretan inhibited sporulation at all tested concentrations. Correspondingly Tilt gave highest record of 98 spores only at 1g/l. Benlate gave 500 spores count at the that same concentration (Table 1), and it allowed sporulation up till the 4g/l fungicide level. Dithane-M45 gave comparatively highest spore count of 1200 spores at 1g/l but this value declined with increasing concentrations to a final count well above corresponding values recorded for other fungicides.

FUNGICIDES	CONCENTRATIONS, g/l	NUMBER OF SPORES PRODUCED
Aretan	1.0	0
	2.0	0
	3.0	0
	4.0	0
	5.0	0
Benlate	1.0	500 ± 70.8
	2.0	320 ± 55.6
	3.0	214 ±36.4
	4.0	126 ± 22.8
	5.0	0 ± 0
Dithane M45	1.0	1200 ±70.8
	2.0	965 ±78.5
	3.0	684 ±54.8
	4.0	566 ± 53.2
	5.0	298 ± 65.8
Tilt	1.0	98 ± 4.3
	2.0	64 ± 3.6
	3.0	55 ± 5.8
	4.0	24 ± 4.3
	5.0	0 ± 0
CONTROL	0.0	1700 ± 120.8

Table 1 : The effect of fungicides at different concentrations on sporulation of *Curvularia cymbopogonis* 10 days after inoculation on potato dextrose agar. Data are means of 4 replicates.
± Standard Error of the mean.
Table 1 : Sporulation de *Curvularia cymboogonis* après 10 jours de culture sur " potato dextrose agar " en présence de différentes concentrations des fongicides étudiés. Les valeurs correspondent à la moyenne de 4 réétitions.
± Écart Moyen.

DISCUSSION

Experimental results show that, Aretan was the most effective in inhibiting spore germination, followed by Tilt, Benlate and lastly Dithane-M45. Olufolaji (1983, 1985) has demonstrated the potency of both Aretan and Benlate in fungal disease control. This is in agreement with results obtained in this study. The mercurial property of Aretan is likely to enhance its highest potency. Dickinson and Wallace (1976) had indicated that a high proportion of the leaf surface mycoflora was susceptible to most fungicides, especially those of wide active spectrum such as Aretan and Benlate. Furthermore the performance of Benlate conformed with previous marks. Benomyl (benlate) wide spectrum of activities against fungi has been already confirmed unlike Zineb and other systemic fungicides and its activities (Waren, 1974). Where some recovery of the microbial populations were observed even after repeated sprays.

Dickinson and wallace (1976) reported that repeated sprays of tridemorph had only minor effects on the phylloplane population of leaf spot pathogens of groundnut when benomyl inhibited the development of many yeasts and filamentous fungi.

With regards to mycelial growth and sporulation, inhibition by Aretan was most effective; it is followed by Tilt, Benlate and lastly Dithane-M45. Growth results obtained in this study, when compared with those of spore germination further confirms that fungicides in general are active at various stages in fungal development, but if applied on time could have a follow up effect. Tilt produced mycelium and no sporulation at the 2g/l concentration and above. It is probable that the active ingridient has caused enough damage in the physiological set-up of the fungus to prevent cell differentiation allowing sporulation.

Since spore formation is the main vehicle of fungal disease dissemination, any fungicide that effectively inhibits sporulation is most promising. In this respect it has earlier been stated that Dithane-M45 allowed sporulation at lower concentration. It is used to control *curvularia* leaf spot disease may thus prove ineffective.

Culbreath *et al* (1993) used Dithane-45 (mancozeb), among other fungicide, in controlling, *Cercosporadium personatum* on *Arachis hypogea* and achieve some degree of success. The fact that our observation do not conform with earlier observation may be due to difference in pathogen characteristics.

REFERENCES

ANTOINE R, 1961 — *in* : *Sugarcane diseases of the world*. Martins. J.P, Abbott E. Vand Hughes. C.S. Ed. Elsevier Publ. Co. Amsterdam, pp 326-354.

CULBREATH A.K., BRENNEMAN T.B., CHANDLER I.D. & SUMMER H.R., 1993 — Chemigation and ground spray applications of cyproconazole for control of late leaf spot of peanut. *Plant Disease*, 77: 505-507.

DICKINSON C.H. & WALLACE B., 1976 — Effects of late application of foliar fungicides on activity of micro organism on winter wheat flag leaves. *Transaction of the British mycological society*, 67: 103-112.

FAJEMISIN J.M. & OKUYEMI O., 1976 — Fungicidal control of Curvularia leaf spot disease of maize. *PANS* 64: 12-14.

OLUFOLAJI D.B. & OLOFINBOBA M.O., 1984 — The control of Smut with fungicides in two sugarcane cultivars in Nigeria. *Sugarcane* 5: 12-19.

OLUFOLAJI D.B., 1985 — *Fungicides combination trials for smut control in Nigeria. Sugarcane* 1: 8-11.

OLUFOLAJI D.B., 1986 — *Curvularia* leaf spot disease of sugarcane: A New Disease. *Sugarcane* 2: 8-11.

OLUFOLAJI D.B., 1993 — Evaluation of some relatively new fungicides for smut control in sugarcane. *Crop Protection 1993*, 12(4): 293-295.

PARRIS G.K., 1950 — The *Helminthosporium* that attack sugarcane. *Phytopathology* 40: 90-103.